Isolator Rodent Caging Systems (State of the Art): A Critical View

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The past 15 years have brought remarkable changes to the way we house rodents used in research. These changes coincided and, to a great extent, were driven by a marked increase in the use of genetically engineered mice. Notable changes included the routine use of isolator cages and more recently, the transition to individually ventilated isolator caging systems. Although many of the newer systems offer considerable advantages, they are highly capital intensive. Importantly, users must recognize the operational differences among various caging systems to select those appropriate to specific applications. In this review, a historical perspective, review of the limited experimental data pertaining to system evaluations, overview of design and operation of commercially available ventilated caging systems (VCS), and review of selection preferences on the basis of the intended application will be provided. The reader should also recognize the extensive degree of change that continues to occur with respect to the design of isolator caging systems, variety of systems offered, and number of vendors manufacturing them.

Static Isolator Caging Systems:
Although today, most institutions using rodents are capable of acquiring and maintaining them free of adventitious agents, this change is recent. It has only been during the past 20 years that commercial vendors have consistently provided rodents free of unwanted infective agents, and institutions have successfully maintained them in that desired "clean" state. The ability of institutions to maintain "clean" rodents, especially when contaminated and clean rodents are housed and used simultaneously, can be attributed, in part, to implementation and use of the modern static isolator caging system.

The first isolator cage, developed by Lisbeth Kraft in the late 1950s (1), was a cylindrical cage manufactured with a solid galvanized bottom and tight-fitting lid with metal mesh sides wrapped with fiberglass insulation that filtered incoming air (Figure 1). The cages, bedding, feed, and water were steam sterilized and were only manipulated in a bacteriologic transfer hood, the precursor of the biological safety cabinet. Using this system, Dr. Kraft and subsequently, other investigators, using similar systems, were able to prevent contamination of immune-naïve mice with epidemic diarrheae of infant mice, a highly infective rotavirus (1–4).

The ensuing years saw the development of a variety of filter lid designs motivated by Dr. Kraft’s success. Lids included molded fiberglass media held in place with a wire retainer, fine wire mesh, and molded polyester. Many of these lids shared the disadvantages of becoming dislodged easily, misshapen during washing or sterilization, and decreasing intracage ventilation.

The modern filter top was the brainchild of Robert Sedlacek, who was charged with oversight of a large gnotobiotic mouse colony used in radiation biology research at the Massachusetts General Hospital (MGH). The economic concerns of maintaining their gnotobiotic colony in isolators was the driving force that led Mr. Sedlacek and his colleagues at MGH to develop the static isolator caging system used extensively in the United States today. This cage design also is used in several VCS. The Sedlacek cage markedly improved static isolator top cage design. The top consisted of a polycarbonate frame fitted with a piece of polyester filter medium held in place by a perforated aluminum plate (5). The rim at the bottom of the filter top where it fit over the underlying cage, formed a lip creating a junction similar to that found in a petri dish. Another component of the system’s success was the concurrent use of a horizontal-flow class 100 mass air displacement unit (MADU), in which mice and steam-sterilized cages, filter tops, bedding, and feed, and mice were manipulated. This system, when used with appropriate procedures, is an extraordinarily effective tool to maintain animals, including immunodeficient rodents, free of unwanted pathogens.

Currently, the Sedlacek filter top is manufactured by several companies in the United States in two forms: a standard top, and a reduced height lid, which permits greater stocking density. The aluminum plate, which has been replaced with a polycarbonate grid, retains a filter usually manufactured from Reemay® fabric. Reemay® fabric is spunbonded polyester fabricated into a sheet of continuous randomly arranged filament polyester fibers, and is the principal medium used in filter tops. Reemay® filters are available in a variety of thicknesses and in two deniers, low and regular, that have different particle arrestance capabilities. Reemay® 2033, 2024, and 2295 are the most commonly used filter top grades in use. The fabric’s ability to pass air is inversely proportional to its particle arrestance capability. The static isolator caging system is capable of protecting product (animals), if used with either a horizontal or vertical airflow class 100 MADU, or product and personnel, if used in conjunction or with a biological safety cabinet (BSC) and appropriate

FIG. 1. The first isolator caging system developed by Lisbeth Kraft consisted of a galvanized wire cylinder with solid bottom and lid (left) that was wrapped with fiberglass filter media (right). From Yale J. Biol. Med. 1958; 81:121–137.
technique. Filter tops also retain allergenic proteins within the cage, reducing their levels in the macroenvironment (6). Because allergy is among the most important occupational disease affecting animal handlers, this advantage is significant (7).

Soon after the Seldacek static isolator caging system came onto the commercial market, users, especially those in temperate regions where dehumidification is difficult during the summer, became aware of the system's principal limitation. The filter top design, which is extremely effective at providing protection to the animals or containing contaminants within the cage, substantially impedes air exchange between the microenvironment, to which the animals are exposed, and the macroenvironment, which is ventilated and rigidly controlled. Although previous filter top designs had been associated with similar problems, the Seldacek design resulted in greater impendence to air exchange, resulting in considerable microenvironmental air quality issues (8–12). Experimental studies conducted in the late 1980s revealed important characteristics of the system. The lid substantially hampers ventilation, as most air exchange between the cage's microenvironment and the room takes place at the cage-lid interface, not through the filter medium, as one may expect (13). The amount of filter medium exposed on the lid's surface played no or minimal role with respect to intracage ventilation (12). The filter top is a substantial barrier to moisture exchange (9, 12, 14–16). Intracage relative humidity (RH) may be increased by as much as 98%, compared with microenvironmental RH, and this difference is more pronounced when housed at lower macroenvironmental RH (15–17). Microenvironmental RH increases with housing density (15, 16). The principal concern resulting from increased microenvironmental RH is its impact on intracage ammonia (NH₃) concentration. Microenvironmental NH₃ concentration may reach 550 ppm within 7 days, depending upon bedding used and macroenvironmental RH, when housing the maximum number of mice prescribed in the Guide (18, 19). This concentration exceeds, by as much as 14-fold, limits (25 ppm 8-h time-weighted average (TWA)) established by the American Conference of Governmental Industrial Hygienists (ACGIH) for human exposure in the workplace (20). Physiologic alterations and interference with research are expected at concentrations that can be observed in static isolator cages (21–31). In addition, humane concerns have been raised because NH₃ is a potent irritant of mucous membranes (32). Interestingly, Bob Seldacek and his colleagues never faced this problem, because the gnotobiotic colony for which they developed the system was free of urease-producing bacteria, which convert urea in urine and feces into NH₃. They also autoclaved the contact bedding, which may contain heat-labile ureolytic and urease-activating enzymes (33).

In addition to NH₃, the concentration of carbon dioxide (CO₂) is also significantly increased in static isolator cages (10, 12, 34). Concentrations can be up to 4,000 ppm higher than those observed in the macroenvironment when housing the maximal permissible biomas (35). However, maximal CO₂ concentration observed in static isolators does not exceed the exposure limits (5,000 ppm 8-h TWA) established by the ACGIH for humans (20). Therefore, little concern has been placed on its increase. However, physiologic alterations that could impact select research investigations are possible.

The search for additional microenvironmental contaminants in static isolators has been limited. Evaluation of the microenvironment in static isolators containing a variety of contact beddings was conducted for hydrogen gas, 2-butanol, acetonitrile, ethyl alcohol, carbon monoxide, acetic acid, hydrogen sulfide, sulfur dioxide, formaldehyde, and volatile alcohols and ketones, all potential products of bacterial enzymatic activity (36). Only an increase in acetic acid (0.86 ppm) and sulfur dioxide (0.42 ppm), concentrations was detected in cages containing autoclaved corn cob bedding. Both compounds were detected at concentrations below their ACGIH 8-h TWA of 10 and 2 ppm, respectively (20). However the acetic acid concentration exceeded NIOSH's TWA of 0.5 ppm (36). Acetic acid was off-gassed, presumably from decay of vegetative material associated with the corn cob, rather than produced from bacteria associated with the mice. In another study, methane and hydrogen sulfide concentrations were evaluated in static isolators (34). Methane was observed at concentrations > 500 ppm after 7 days; however, an increase in hydrogen sulfide concentration was not detected. The physiologic relevance of these findings and their effects on research were unclear. Experimental evidence exists that uncharacterized pollutants exist in isolator cages. A study on the inhalational toxicity of methyl bromide in rats revealed olfactory sensory cell loss that could not be attributed to the high NH₃ concentration observed or methyl bromide exposure (37). However, the presence of only NH₃ and hydrogen sulfide was examined.

Bae et al. (38) recently observed that the growth rate of mice housed in static isolators for 1-week periods was significantly greater than the growth rate of mice when housed in cages without a lid. Because food and water consumption were not affected, the authors speculated that the differences could be attributed to a change as simple as a decrease in animal activity when mice are housed in the warmer and more humid microenvironment of static isolators, or another more complex regulatory mechanism.

The National Institutes of Health's (NIH) Department of Engineering Services recently published results from an in-depth study modeling micro- and macroenvironmental conditions associated with static isolator cages maintained in numerous animal holding room configurations. The study involved use of empiric measurements, wind tunnel testing, and notably, computational fluid dynamics (CFD) (39), which is an advanced 3-dimensional mathematical model that is used to predict the movement of air, water, or any other gas or liquid. This study represents the first use of CFD in laboratory animal facility and cage evaluation in which the boundary conditions necessary for accurate CFD computations were determined and experimentally validated. The NIH study indicated that many previous assumptions made with respect to room configuration, particularly the locations and types of supply diffusers and exhaust register locations, and their effects on the static isolator cage microenvironment, may be contrary to previously accepted tenets. Room configurations providing improved microenvironmental conditions for the animals may be distinctly different from those used to improve macroenvironmental conditions for personnel. For example, although high-level exhausts improve thermal ventilation efficiency, they reduce intracage ventilation in static isolator cages and lead to increases in microenvironmental NH₃ and CO₂ concentrations.

Static isolators, despite their disadvantages, play an important role in animal research today. They are particularly useful for studies where containment at the cage level is desirable (40). Examples include in vivo administration of hazardous agents, such as biological pathogens, hazardous chemicals, and radionuclides. Static isolators can be placed in a secondary enclosure such as a negative flow MADU, a BSC, or a chemical fume hood for an additional level of containment. When used for hazardous agent containment, static isolators should preferably be opened, and the contaminated animals and cage contents handled, in an appropriate BSC or fume hood, dependent on the hazard used. Intracage ventilation and, as a result, microenvironmental conditions, improve when static isolators are housed in a MADU, presumably because of increased air flow over the filter top (17).

Static isolator cage users should be aware of the system's limit-
tations, and of the methods available to address them. There are four principal ways of addressing poor microenvironmental air quality, notably accumulation of intracage NH₃ concentration: change cages at sufficient frequencies (17); use a contact bedding with desirable performance characteristics (35); reduce macroenvironmental RH (12); and increase macroenvironmental temperature without altering moisture content in the air (39). Cages should be changed before intracage NH₃ concentration accumulates. Figure 2 contains a typical NH₃ generation curve from a static isolator containing autoclaved pine shavings housed in a room at 21°C and 44% RH. In this example, if cages were changed twice weekly, mice would not be subjected to high NH₃ concentration, because NH₃ concentration does not begin to increase until day 4. Significant differences in the NH₃ generation curve are observed when different contact beddings are used (35). Two principal differences are observed: the day on which NH₃ is first detected, and the slope of the curve. For example, microenvironmental NH₃ concentration is not generally detectable during a 7-day change cycle, at macroenvironmental temperatures and RH values prescribed in the Guide, when housing most strains of mice on contact bedding with desirable characteristics (e.g., corn cob). In contrast, NH₃ concentration may be markedly high in cages situated under identical macroenvironmental conditions containing the same strain and numbers of mice when containing less desirable bedding (e.g., pine shavings). As illustrated in Figure 3, the NH₃ generation curve can be shifted to the right by selecting a bedding with desirable characteristics. This delays onset of NH₃ detection, as well as decreases the curve's slope, so that less NH₃ is generated over unit time. A similar result can be attained by decreasing macroenvironmental RH while using the same bedding. A macroenvironmental RH, bedding-dependent, threshold exists over which the NH₃ generation curve is similar among beddings (41). When macroenvironmental RH is > 70%, the NH₃ generation curve is similar between corn cob and pine shaving beddings. Lowering macroenvironmental RH to 60% reduces the slope of the corn cob bedding NH₃ generation curve to near zero over a seven-day period, whereas the pine shaving NH₃ generation curve remains unchanged. Further reduction of macroenvironmental RH to < 50%, will reduce the slope of the pine shaving NH₃ generation curve to near zero, resembling the corn cob bedding curve at 60% RH. The specific characteristics of bedding responsible for these differences are unknown; however, it is not simply related to absorbency (35). Bedding types can be mixed to take advantage of desirable characteristics offered by different beddings.

It is essential to understand the relationship between NH₃ generation, bedding, and macroenvironmental RH, especially when conducting and interpreting experimental studies. Choi et al. (16) evaluated the effects of housing density on intracage RH and NH₃ concentration in static and ventilated isolator cages containing corn cob bedding in a macroenvironment with 43 and 39% RH, respectively. The authors did not detect NH₃ in ventilated isolator cages 32 days after changing, and NH₃ concentration was < 4 ppm on day 10 in static isolators, both housing up to 4 adult mice. The results of this study likely would have been considerably different and perhaps would have yielded a different interpretation had this study been conducted by use of a different contact bedding and at a higher macroenvironmental RH. In another study, Reeb et al. (42) examined the impact of macroenvironmental ventilation rates on microenvironmental NH₃ concentration and RH; however, macroenvironmental RH was not kept constant, but decreased from 50 to 22% RH as ventilation was increased. Conclusions from this study were called into question (43).

These illustrations indicate frequency of cage and bedding changes should be performance based. Microenvironmental NH₃ concentration is the single most important criteria that should be used for this determination in most rodent colonies. Therefore, the author recommends that facilities housing rodents should periodically measure intracage NH₃ concentration. Fortunately, it is easy and inexpensive to make such determinations. Dosimetry systems, such as the Matheson-Kitagawa Toxic Gas Detection System (Matheson Gas Products, Secaucus, NJ) or the Draeger Multi-Gas Detector System (Draegerwerk AG, Lubeck, FRG), use a manually operated air sampling pump and detector tube. Because removal of the filter top to measure NH₃ concentration markedly affects its intracage concentration, sampling cage(s) should be modified with ports, so that intracage NH₃ concentration can be routinely measured.

**Ventilated isolator caging systems:**

Room-coupled ventilation systems, used with static isolator cages, achieve microenvironmental ventilation passively by diffusion, convection, and radiation. Numerous studies have indicated that this method, which is energy and operationally intensive, is ineffective (8–12). In contrast, the microenvironment is ventilated directly in cage-coupled systems, vastly...
enhancing microenvironmental air quality. The improvement in microenvironmental air quality obtained by use of VCS is one of the principal reasons for their intense growth in popularity.

The earliest documented attempt to directly ventilate rodent cages originated from the Jackson Laboratory (Jax) in the 1960s (44, 45). Under the direction of Dr. Ed Les, Jax initiated the development of a VCS, not only to improve intracage ventilation, but also to increase housing capacity. Jax developed several prototypes before collaborations were initiated with Thoren Caging Systems, Inc. in the late 1970s. The original system developed for Jax was modified in the early 1980s into the first commercially available VCS. A subsequent iteration of the system, which is commercially available, incorporated the use of a Recmay®-containing filter top, which was not present in the earlier model.

Spurred by existence of poor microenvironmental air quality in static isolator cages and its potential effects on cage occupants, VCS gained widespread popularity by the early 1990s. This remarkable growth could also be accounted for by the burgeoning expansion in mouse populations at many institutions, a result of increased production and use of unique transgenic and "knock-out" strains. The number of commercial systems available from which to choose expands annually. Existing manufacturers have continued to refine and expand available models and options. Unfortunately, with the proliferation of systems, it has become extremely difficult for users to determine which system(s) best meets their needs and offers the necessary flexibility, especially in light of limited comparative data, lack of agreement on suitable ventilation rates, and absence of standardized techniques to evaluate system performance.

Ventilated isolator caging systems offer a number of advantages over static systems. It has been clearly demonstrated that these systems markedly improve microenvironmental air quality (16, 19, 54, 46-50). The intracage concentration of NH₃ (Figure 2) and CO₂ are considerably lower in ventilated cages, compared with static cages, housed under the same macroenvironmental conditions with the same strain and biomass of mice. Further, the day on which NH₃ is first detected is delayed in ventilated cages. Not only is the intracage air quality improved, but also the variability in microenvironmental air quality observed among static isolator cages housed in the same macroenvironment is reduced or eliminated in VCS (19). Because less NH₃ is generated in ventilated cages, macromolecular air quality should improve for personnel working in animal holding rooms and cage wash.

In most facilities, depending on the strains of mice housed, their experimental use, housing density, and institutional perspective, ventilated cage changing is delayed to weekly or even longer (51). In contrast to static isolator cages, which frequently require changing twice weekly, once-weekly cage changing translates to considerable labor savings. In addition to the labor savings in changing cages, the time spent sanitizing cages and the quantity of bedding used also is reduced. The longevity of cage components, especially those made of polycarbonate that must be autoclaved, is increased. All these factors, dependent on the facility and rodent population, may translate to substantial operational savings.

Another appreciable advantage offered by VCS is the opportunity to substantially increase stocking density. When contrasted to static caging systems (SCS) occupying the same footprint, VCS can house up to 100% more mice, dependent on which systems are compared. A VCS occupying a footprint of < 2.5 X 6 ft can house as many as 140, 11.5 X 7.25-in rodent shoebox cages. Clearly, VCS can be used to markedly enhance housing capacity in facilities previously using SCS. Alternatively, use of VCS may permit institutions to substantially decrease the size of a new facility or permit conversion of existing rodent housing space to other uses. Under these circumstances, cost savings can be substantial, not only in construction savings, but also reduced operational costs, which can be considerable. The utility costs necessary to provide HVAC to animal facilities are estimated at $2.50 to 4.00 ft³/min (CFM)/yr in the United States (52). If a 30,000-gross ft² (gsf) animal facility with animal holding rooms ventilated at 15 air changes/h is reduced by 10,000 gsf by using VCS, the energy savings alone is estimated at $50,000.00 to $80,000.00/yr. Giving consideration to additional operational, construction, and labor savings, the premium paid for purchasing VCS, compared with SCS, can be quickly recovered.

VCS offer additional advantages that may be applicable under particular circumstances. They can be used in a rodent holding room(s) or a facility with inadequate ventilation in lieu of the more expensive option of replacing or expanding an existing HVAC system. VCS ventilate cages directly and more effectively. Micro- and macroenvironmental performance standards can be achieved with considerably less supply air than necessary when using SCS. Because the microenvironmental air volume is smaller than that of the macroenvironment, the microenvironment can be ventilated at higher rates using less supply air than is needed to ventilate the macroenvironment.

VCS can be integrated into facilities by use of a variety of methods dependent on whether the supply air is provided directly from, or exhaust is exhausted directly into, the building's HVAC system. For example, if the exhaust from VCS is exhausted directly into the facility's exhaust system, the supply air volume provided to the room can be reduced considerably under most conditions. This method of installation requires lower macroenvironmental ventilation rates, reducing energy costs. These concepts have been reviewed in detail (53).

VCS can also provide, dependent on the specific system used, an additional protective barrier to animals housed within the cage (54, 55). Systems pressurizing the cage with HEPA-filtered supply air provide cage occupants an additional level of protection from contamination. The effectiveness of VCS has been documented experimentally (55). Immune-naïve mice, seronegative to mouse hepatitis virus (MHV), were housed in uncovered shoebox, static isolator, and ventilated isolator cages in the presence of uncovered cages containing mice that were infected with and shedding MHV. Cages were autoclaved and only manipulated within a class-II BSC. Whereas 100 and 20% of immune-naïve mice housed in uncovered shoebox and static isolator cages, respectively, seroconverted to MHV, none of the mice housed in positively pressurized ventilated cages seroconverted.

Because many VCS filter (HEPA) the cage exhaust before releasing effluent into the room, or alternatively, directly exhaust exhaust into the building's HVAC system, the concentration of allergenic particulate in the macroenvironment may be reduced. Macroenvironmental particulate concentrations have been evaluated in a single VCS (56). Particulates, detected by use of settle plates, were reduced 99 and 94%, compared with those in open-top cages when the system was operated in positive or negative modes, respectively. It is important to note that comparison was not made with static isolator cages, and only one VCS type was evaluated. Use of other systems may result in higher concentrations of particulates released into the room. Several common VCS operate by pressurizing the cage, attempting to capture cage effluent after it escapes from the cage. Leakage of cage effluent into the macroenvironment has been detected by these systems, using a tracer gas (57).

Although the advantages of VCS are clear, there are several important considerations when selecting or using these systems. The user must clearly understand the operating principles of the system they intend to use. Specific systems differ with respect to the method of introduction and quantity of air supplied to each cage. The ideal intracage ventilation rate for VCS is un-
known and is likely dependent on numerous factors, including species, strain or stock housed, cage population, and bedding used. An ideal rate in one situation may be insufficient or excessive in another. The criteria used to select intracage ventilation rates should be based on performance standards. The author recommends that ventilation rates be established in VCS so that, prior to cage changing, microenvironmental NH₃ and CO₂ concentrations are < 25 and 5,000 ppm, respectively, and temperature and RH fall within the limits prescribed in the Guide (18). Further, Intracage air speed, at locations cage occupants would expect to encounter, should be < 50 linear feet/min (1.5 m/min), a rate considered to be still air in human environments and unlikely to cause appreciable physiologic effect in most species (58, 59). Results of a recent study conducted at Jax documented that Intracage ventilation rates should be increased from 60 to 100 air changes/h when housing breeding trios and pups in lieu of adult males if the same changing frequency and Intracage NH₃ concentration are to be maintained (51). It is extremely difficult to obtain accurate information on ventilation rates for VCS. The technology available to measure ventilation rates is designed for evaluating rooms or buildings; it is not designed to accurately evaluate enclosures the size of rodent cages, which have a volume < 1 ft³, or whose air supply or exhaust rates may be < 0.5 CFM. Several groups have used tracer gas (SF₆) decay to evaluate air exchange rates in ventilated cages (51, 56, 57). Although it is more accurate than other techniques, the small cage volume limits accuracy (57).

Excess intracage ventilation, especially when air is supplied at the level of the cage, may lead to chilling and dehydration, especially of neonates and hairless mutants. The speed of air to which animals are exposed affects the rate at which heat and moisture are removed from an animal. Air at 20°C moving at 60 fpm has a cooling effect of approximately 7°C (14). It may be necessary to increase microenvironmental temperature when housing animals in VCS with high intracage air velocities, when housing neonates, hairless mutants, or single animals, or when contact bedding is unavailable or is a type that does not provide the animal the ability to nest. Pheromone dilution may also be problematic when breeding particular rodent species, stocks, or strains. Huerkamp et al. (60) have documented a negative synergistic effect between ventilated cages and use of automatic watering systems leading to increased mouse pup mortality. Further, they reported that pups reared in VCS were smaller than those reared in SCS and attributed the change to intracage ventilation.

There are considerable differences in VCS ventilation rates on the basis of the manufacturer, system type, and even the age of the system. Comparison of 3 commercial systems indicated that intracage ventilation differed by as much as 88% (57). It is also notable that velocities exceeding 50 fpm were detected in 2 of 3 VCS evaluated, with speeds approaching 100 fpm detected in one system (57). Until recently, there was a trend for manufacturers to increase intracage ventilation rates with newer VCS models; however, adverse effects reported by users have many manufacturers reconsidering this strategy. Fortunately, ventilation rates can be adjusted in most VSC by adjusting exhaust and/or supply fan speeds or dampers.

Additional considerations when using and selecting VCS include heat load, noise generation, power requirements and failure, vibration, and sanitation. Because VCS enable users to increase stocking density by up to 100%, the heat load generated by the animals may be of considerable magnitude. The heat load generated by the supply and exhaust blowers when combined with the animals' thermal load, especially in holding rooms with marginal temperature control, may exceed the HVAC system's cooling capacity. Frequently this issue can be resolved by directly venting the VCS exhaust into the building's HVAC system, because much of the thermal load is contained within the exhaust effluent.

The VCS blowers generally use 110-V current. Dependent on the system's design, the exhaust and supply blowers may be interconnected, requiring a single outlet, or each blower (supply and exhaust, if equipped) may require its own. When installing multiple VCS racks, the number of electrical outlets required may exceed the available capacity. It is prudent to place VCS on circuits served by electrical generators, because the design of many VCS does not provide the capability for passive ventilation in cases of power failure. In fact, some systems use solid tops, without filters, and attach firmly to the cage below with a gasket and/or clips. It may be critical in select installations to ensure that exhaust and supply blower operation are interconnected functionally so that, if one fails, the other is automatically disabled. For example, if it is critical that the VCS provides product protection, then supply blower failure must be accompanied by exhaust shut down. If this feature is not used, cages will develop negative pressure if the supply blower fails or its output is diminished. Most systems are available with warning lights, magnathelic gauges, and audible and/or voltage alarms that require either active or passive monitoring by facility staff.

Noise generated by exhaust and/or supply blowers is a consideration dependent on system type and the number of VCS units maintained per holding room. Noise must be addressed from two perspectives: the effect(s) of macroenvironmental noise on personnel servicing and working within the holding room; and the effect(s) of microenvironmental noise on the cage occupants. The impact of noise on animal behavior and physiology has been described (61, 62). Rodents' hearing range overlaps, only partially, with that of humans; their range extends to ultrasonic frequencies not heard by people (63). Limited data have been reported on noise generated by VCS (19, 56). Micro- and macroenvironmental noise at frequencies between 31.5 and 16,000 Hz were evaluated in three commercial VCS (19). All three systems produced room noise that was significantly higher than room background. One unit generated more noise (80 dB) than did the other two units (74 dB) evaluated. Recognizing that the dB scale is logarithmic, this difference is discernible. Microenvironmental noise was found to be higher at lower frequencies, compared with macroenvironmental noise and noise generated at higher frequencies, in the three systems evaluated. The relevance of these findings for rodents was unclear. It is speculated that rodents have a higher tolerance for low-frequency than they do for high-frequency noises (64). However, the authors did not evaluate ultrasonic frequencies. Ultrasonic frequencies were not detected in another evaluation of a single VCS (56). A logarithmic equation is used to determine the increase in dB levels when additional units generating equal amounts of noise are placed in a room. There is an increase in 3 dB with the second, 1.8 dB with the third, 1.2 dB with the fourth, and <1 dB for each successive unit added (65). Therefore, in a room containing 4 units generating 80 dB each, the room noise level would be 86 dB, a level above the ACGIH-established 8-h TWA of 85 dB (20).

The physiologic effects of continuous low-level vibration have not been investigated to the author's knowledge. The VCS with blowers attached directly to the rack are more likely to generate vibration at the cage level. Whereas behaviorists are concerned about environmental stimuli provide timing cues, VCS units are operated continuously; therefore, they would unlikely have any effect in this regard. Regardless, system manufacturers have taken some or all of the following steps to reduce or eliminate intracage vibration, including: placing rack-mounted blower housings on rubber and/or spring-loaded mounts; housing blowers on a rack/shelf separate from the caging; using flexible plastic hose connectors between the rack air distribution system and the blowers;
and/or using the facility's HVAC system to provide supply and exhaust air.

Because VCS have extensive air distribution systems, a VCS is considerably more difficult to sanitize than a standard shelf rack. In general, blowers, shelves, and/or access panels must be removed and/or opened before placing a VCS in a rack washer. Access to all plenums and ducts on the cage rack may not be possible with every system. Extensive washing by hand is frequently required; the air distribution system may not be sanitized adequately in a rack washer because of limited access to the washer spray. There is no consensus on the sanitization frequency for VCS racks. Racks are broken down and sanitized at least every 6 months at the author's institution, unless there is a change in the animals' health status or special circumstances dictate more frequent sanitization. Prefilters, if supplied on VCS, may require changing or cleaning more frequently, dependent on the specific system and bedding used. The blower units must be disassembled for cleaning because specific components, including the fan motor and HEPA filter, cannot be sanitized by use of liquid. If sanitization of these components is required, gas agents may be used. Although it is labor intensive, VCS can be decontaminated in situ by bagging the entire unit or isolating the holding room and sterilizing, using gas sterilants such as paraformaldehyde.

Because of the difficulty in sanitizing VCS and the potential for unfiltered cage effluent to be released from some systems, considerable thought must be given to their use before housing animals infected with or exposed to hazardous agents. The release of unfiltered cage effluent into the macroenvironment, which has been documented for some systems, raises an additional concern when using hazardous agents (57). The use of VCS to house animals during studies with hazardous agents should be limited to systems that do not release cage effluent into the macroenvironment without first passing it through an appropriate filter and/or releasing exhaust directly into the building's HVAC system. Several VCS can be operated with negative intracage pressure and, therefore, are preferable for these studies. Systems dependent on HEPA filters for safety should be certified for filter integrity and function by certified technicians no less than annually, or more frequently as conditions dictate, as recommended for a BSC (66). Importantly, not all VCS are constructed to permit easy access to test the HEPA filters.

Seven manufacturers currently produce VCS for the US market. There are considerable operating and design differences among these systems. I have categorized VCS into 2 principal classes on the basis of their operating design: intracage supply/perimeter capture; and intracage supply/intracage exhaust systems. The latter group can be further divided into direct, indirect, or combination subtypes dependent on whether the supply or exhaust air passes through a filter, at the level of the cage, before entering or exiting the cage. Figures 4A-F provide schematic representations of the various systems.

Intracage supply/perimeter capture systems are manufactured by Allentown Caging Equipment Co., Inc., Allentown, NJ (ACE) and Laboratory Products, Inc., Seafood, DE (LP). Their systems, the Micro-Flo/Micro-Vent (ACE) and Microisolator VCLTM (LP), respectively, operate by use of similar principles. The HEPA-filtered air is supplied directly, at the level of the cage, resulting in its pressurization. Cage effluent escapes principally at the filter top/shoebbox cage interface and is captured at the interface (ACE and LP) and the filter (LP) by a side-separated U-shaped channel (ACE) or a canopy (LP). These systems can only be operated in the positive-pressure mode. Both systems require a supply and exhaust blower, which can be installed on the cage rack or wall. Alternatively they can be supplied and exhausted by the facility's central HVAC system. Select independent experimental evaluations of both systems have been published (16, 19, 34, 57, 60).

Intracage supply/intracage exhaust (direct) systems are manufactured by Alternative Design, Siloam Springs, AR (AD); Tecniplast S.ar.l., Bugugiate (VA), Italy (TP); and Lenderking Caging Products, Millersville, MD (LK). Their systems, the Gentle-Air (AD), the Tecniplast IVCTM (TP), and the CCM IsoVentTM (LK) supply air directly to the isolator cage lid (AD, TP, and LK) or cage bottom (TP), and exhaust air directly from the lid (AD and TP) or from a plenum beneath the cage (LK). AD's system uses a single blower to provide supply air and remove exhaust. The blower is mounted on either the bottom or top of the rack, dependent on whether the system is purchased to operate in the positive- or negative-pressure mode. Supply and exhaust air flow into and out of the cage through valves that penetrate the solid lid through spring-loaded doors. TP manufactures several versions of their system. Their Vent-Plus IVCTM system supplies air directly to the cage and exhausts air directly from the lid; their SealSafe IVCTM supplies and exhausts air directly to the filter top, which contains a baffle to direct air within the cage. TP's systems can be operated in either positive- or negative-pressure modes by electronically altering the quantity of supply and exhaust air by adjusting blower speed.

LK's cage is divided into three sections, a supply air plenum created by a solid top and feeder, the cage body containing the animals with a perforated floor, and the waste compartment beneath the cage into which excrement falls and exhaust is evacuated. Alternatively, solid bottom cages or a filter top can be substituted for the perforated cage or solid lid. Blowers can be mounted directly on the rack (AD, TP, and LK), on a separate rack (TP and LK), or may be wall mounted (AD, TP, and LK). To the author's knowledge, independent studies of these systems have not been published. All three systems (AD, TP, and LK) can be operated off of facility HVAC in lieu of the unit blowers.

Intracage supply/intracage exhaust (indirect) systems are manufactured by Thoren Caging Systems, Inc., Hazelton, PA (TCS) and BioZone, Margate, Kent, UK (BZ). Their systems, the Maxi-Miser ITTM (TCS) and the VentiRackTM (BZ), provide supply air and remove exhaust, through a filter in the cage lid that resides directly below a positive and negative plenum or duct. Supply air diffuses from the plenum or duct through the filter into the cage while the reverse occurs for exhaust. Both systems can be operated in a positive- or negative-pressure mode by either altering the position of dampers manually (TCS), or electronically altering the quantity of supply and exhaust air by adjusting blower speed and/or the pressure decrease across control valves located in the supply and exhaust ducts (BZ). Blowers can be mounted either directly on the rack (TCS and BZ), directly beneath the rack on an independent sled (TCS and BZ), on a separate rack (TCS), or on the wall (TCS and BZ). Alternatively, facility HVAC can be used to provide either supply, exhaust, or both (TCS). Both systems (TCS and BZ) can be installed to direct exhaust effluent directly into the facility HVAC system. The TCS system has been independently evaluated experimentally by several investigators (19, 54, 51, 56, 57).

Intracage supply/intracage exhaust (combination). TCS offers an optional valve(s) on their isolator top that is actuated when the cage is placed on the rack and closed when the cage is removed. The valve, which is generally placed on the supply, provides direct inflow of air, circumventing the filter in the lid, maintaining intracage positive pressure. ACE recently introduced a bio-containment unit (BCU) VCS. The BCU contains a shoebox cage into which HEPA-filtered air is supplied, and a solid locking lid with a perimeter gasket that contains a filter through which air is exhausted. The TCS system has been experimentally evaluated (56).

Many factors should be considered when selecting a VCS. Because these systems are expensive, selection of an appropriate system is essential. Considerations, some of which have been dis-
FIG. 4 (A-F). Schematic representations of types of commercially available ventilated caging systems. Notice that all systems are shown with automatic watering. Vectors representing air flow are shown for illustrative purposes only and do not necessarily reflect air flow patterns within the cage. Numbers in brackets reflect the 5 commercially available versions of the intracage supply/intracage exhaust ventilated caging systems.

cussed in this review, include: use of the system; importance of product versus personnel protection; desire to alter the system’s operation from positive to negative pressure or vice versa; cost, including replacement costs for cage components with limited life; serviceability and service support; ease and ability to sanitize; microenvironmental control; animal visibility; micro- and macroenvironmental noise; ability to evaluate filter (HEPA) function; ventilation method and rates; method of facility integration; and weight and mobility.

Unfortunately, it is difficult to compare operational issues such as ventilation rates, microenvironmental control, and containment capabilities, because only limited studies of select systems have been published, using techniques that have not been standardized. Objective criteria, such as those developed and
published to ensure functional criteria of BSCs (67), must be developed to evaluate VCS. The VCS manufacturers, engineers, safety specialists, and VCS users must come together and begin to address this critical issue.

It should be clear from this review that facility managers, veterinarians, and scientists, now more than ever before, have a variety of caging systems from which they can choose to house laboratory rodents. It is critical that individuals responsible for selecting caging systems for purchase, as well as system users, understand the functions and limitations of the system(s) they use.

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This issue features a Special Topic Overview paper from Dr. Nell Lipman, "Isolator Rodent Caging Systems (State of the Art): A Critical View". His paper was solicited following a seminar presented at the 48th AALAS National Meeting in Anaheim, California in 1997. Dr. Lipman, a long time advocate of ventilated caging systems and a leader in testing hypotheses about caging through experimental methods, has presented a comprehensive and balanced review of the use of both static and ventilated isolator systems. Dr. Lipman’s interest and expertise in this area is longstanding as evidenced by the many papers which he has authored in the area of rodent caging. Whether you attended the seminar or not, I am confident that you will find his overview timely and extremely valuable. This paper will be widely used as a reference and for decision-making in our field.

Editors, like authors, sometimes have strong opinions. Unlike Dr. Lipman, I remain unconvinced that the advantages of ventilated cage systems outweigh the disadvantages. Improvements in the cage environment, increased stocking density within animal rooms, reduced labor costs and improved biosecurity are the major reasons cited for utilizing ventilated cages. However, as Dr. Lipman indicates, intracage ammonia and humidity in static caging can be managed with optimal bedding choices (autoclaved corn cob bedding), controlled room humidity, and an appropriate change interval. Given these points, I question the relative advantage of these units in improving the intracage environment. Ventilated cage units of 140 cages per rack can indeed maximize capacity. However the use of low-rise static isolation cages on mobile, readily sanitizable 7-shelf racks provides 112 cages per rack and can cost-effectively provide high density housing. If ventilated cages are changed only once per 14 days, they unquestionably reduce labor costs, however cage changes do provide an enhanced opportunity for animal observation as the animals are transferred between cages. I believe the benefits of more frequent animal handling may balance the additional labor costs. Finally, in my opinion, biosecurity of the systems themselves is approximately equal. Problems in biosecurity, and violations of ideal practices, are much more likely due to human error than to mechanical system failure.

What do you think? I invite further discussion of this question and solicit your opinions. Letters can be directed to me at AALAS, 9190 Crestwyn Hills Drive, Memphis, TN 38125.

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